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Genetic variation of two species with different life-history traits in the endangered renosterveld of South Africa – a comparative analysis of *Eriocephalus africanus* and *Hemimeris racemosa*

Steffen Heelemann[1], Veronika Bäuerlein[1], Cornelia B. Krug[2], Karen J. Esler[3], Peter Poschlod[1] and Christoph Reisch[1]*

[1]*Institute of Botany, University of Regensburg, 93040, Regensburg, Germany,* [2]*Laboratoire d'Ecologie, Systématique et Evolution (ESE), UMR CNRS 8079, Université Paris-Sud 11, 91405, Orsay Cedex, France and* [3]*Department of Conservation Ecology & Entomology and Centre for Invasion Biology, Stellenbosch University, Stellenbosch, 7602, South Africa*

Abstract

We tested the effects of life-history traits on genetic variation and conducted a comparative analysis of two plant species with differing life-history traits co-occurring in the highly endangered renosterveld of South Africa. We selected eighteen renosterveld remnants with varying degrees of size and isolation where populations of the herbaceous, annual and insect-pollinated *Hemimeris racemosa* and the shrubby perennial and both wind- and insect-pollinated *Eriocephalus africanus* occurred. We postulated a lower genetic variation within populations and increased genetic variation between populations in the annual than in the perennial species. Genetic variation was lower within populations of *H. racemosa* than within *E. africanus*, as is typical for annual compared to perennial species. Variation within populations was, however, not correlated with fragment size or distance in either of the two species and genetic variation between populations of the two species was comparable (Φ_{ST} = 0.10, 0.09).

Key words: amplified fragment length polymorphism, Cape Floristic Region, Cape lowland, fynbos Biome, landscape genetics

Résumé

Nous avons testé les effets des caractéristiques du cycle vital sur la variation génétique et nous avons mené une analyse comparative de deux espèces végétales ayant des caractéristiques de cycle vital différentes qui cohabitent dans la région très menacée du renosterveld sud-africain. Nous avons sélectionné 18 vestiges de renosterveld dont la taille et l'isolement sont différents et où se trouvent des populations de la plante herbacée *Hemimeris racemosa*, annuelle et pollinisée par des insectes, et de *Eriocephalus africanus*, arbuste pérenne pollinisé par le vent et par des insectes. Nous avons fait le postulat d'une variation génétique plus faible au sein des populations et plus élevée entre les populations chez l'espèce annuelle que chez l'espèce pérenne. La variation génétique était plus faible au sein des populations de *H. racemosa* que chez celles d'*E. africanus*, typique dans la comparaison d'espèces annuelles et pérennes. La variation au sein des populations n'était cependant liée à la taille du vestige ou à son éloignement pour aucune des deux espèces, et la variation génétique entre les populations des deux espèces était comparable (Φ_{ST}=0,10, 0,09).

Introduction

Several problems with studies of genetic variation of plant populations exist, as recognized by Ouborg, Vergeer & Mix (2006), who called for more research on the 'rough edges of conservation genetics'. First, very few population genetic studies on plant species apply multispecies approaches (Young, Merriam & Warwick, 1993; Mix *et al.*, 2006) to investigate species with different life-history traits in the same fragmented landscape (Apararcio *et al.*, 2012).

*Correspondence: E-mail: christoph.reisch@biologie.uni-regensburg.de

Secondly, most genetic studies focus on population sizes and compare genetic variation of observed plants with meta-studies (Hamrick & Godt, 1996; Nybom & Bartish, 2000), whereas the aspect of isolation due to fragmentation is often neglected. Both negative effects (Schmidt & Jensen, 2000) and neutral effects (Young, Merriam & Warwick, 1993) can be observed in this regard.

It is well known that genetic variation strongly depends on the life-history traits of the particular species. Higher genetic variation within populations is found in long-lived, woody, outcrossing and late-successional species (e. g. perennials) compared to short-lived, nonwoody, self-compatible and early-successional species (e. g. annuals), which are also characterized by higher genetic variation between populations (Hamrick & Godt, 1996; Nybom & Bartish, 2000). Both genetic variation within and among populations depended with increasing strength on the lifespan and the mating system of the species (Reisch & Bernhardt-Römermann, 2014). However, little information is available on whether species with different life-history traits are also affected differently by fragmentation (Apararcio *et al.*, 2012).

Renosterveld of South Africa is a species-rich but highly fragmented and endangered mediterranean-type shrubland in the Cape Floristic Region (Rebelo *et al.*, 2006). Renosterveld once filled large proportions of the south-western Cape lowlands (Kemper, Cowling & Richardson, 1999) and mainly agricultural land-transformation destroyed 92% of its former extent (Rebelo *et al.*, 2006; von Hase *et al.*, 2003b). Within this setting, we analysed the genetic variation of two typical renosterveld species with different life-history traits: the herbaceous, annual and insect-pollinated *Hemimeris racemosa* (Scrophulariaceae) and the shrubby, perennial and both wind- and insect-pollinated *Eriocephalus africanus* (Asteraceae).

We postulated that genetic variation differs between populations of annual and perennial species and performed an AFLP (amplified fragment length polymorphism) analysis to detect genetic variation.

Material and methods

Species description

It was not an easy task to find species that occured at all fragments. Therefore, we selected two typical renosterveld species, which can also be found in other parts of the Cape Floristic Region.

Hemimeris racemosa (Houtt.) Merrill (Scrophulariaceae) is widespread throughout the Cape Floristic Region of South Africa and found in high abundances on sand and clay soils (Goldblatt & Manning, 2000). It flowers from July to October and is characterized by double spurred and axillary yellow flowers between 7 and 13 mm in diameter. A stylar polymorphism is described (Pauw, 2005). *H. racemosa* is an outcrossing and oil-secreting specialist, pollinated by oil-collecting female bees of *Rediviva* spp. and pollen-eating beetles of the tribes Nitidulidae, Melyridae and Scarabaeidae (Steiner & Whitehead, 2002; Pauw, 2004, 2005).

Eriocephalus africanus L. (Asteraceae) is endemic to South Africa and many synonyms exist for this species (Müller, Herman & Kolberg, 2001). Locally, it is known as 'wild rosemary' or 'Cape snowbush'. *E. africanus* is a perennial erect shrub, with a branched and cone-shaped habit, growing up to 0.3–0.9 m height and 4 m diameter and a very variable morphology. Leaves are digitiform, and mostly opposite, greyish in colour and often covered with hairs. The heterogamous inflorescence contains central tubular and marginal ligulate flowers found in a terminal or lateral umbelliferous raceme. Ligulate flowers are female and vary from white to purple colour. The red-purple tubular flowers are pseudohermaphrodite with sterile ovary and five anthers. Stylus is unbranched with hairs. The species is wind and insect pollinated. Flowering time in the study region is from July to September, when dense white long hairs develop at the receptacle.

Study design and sampling procedure

For the purpose of our study, we selected eighteen fragments differing in size and degree of isolation in the Swartland Shale and Granite renosterveld (Rebelo *et al.*, 2006), situated up to 40 km north and east of Cape Town, where both *Hemimeris racemosa* and *Eriocephalus africanus* occurred (Table 1). It was difficult to find still existing renosterveld patches in the focus area. However, we tried to sample all visible fragments in this area (using binoculars, GIS, aerial photographs). In each population, leaf material from seven to 20 individuals was collected and cooled on ice. Leaves were later placed into filter bags and dehydrated in silica gel. Population size of the two species was estimated as fragment size. In total, leaf material of 292 *H. racemosa* and 304 *E. africanus* individuals was collected.

Table 1 Sampled populations of *Hemimeris racemosa* and *Eriocephalus africanus* (Pop.: Population number, Long.: longitude East, Latit.: latitude South, Size: fragment size in ha. Data following von Hase *et al.* (2003a) and estimations from aerial photographs)

Pop.	Location	Long.	Latit.	Size
1	Tygerberg	18°35′39″	33°52′37″	595
2	Kanonkop	18°36′16″	33°49′35″	78
3	Koeberg	18°33′28″	33°42′49″	141
4	Porquepines	18°35′15″	33°46′10″	248
5	Meerendal	18°37′23″	33°46′59″	298
6	Sondagsfontein	18°39′44″	33°45′50″	78
7	Koopmankop 1	18°45′55″	33°54′14″	281
8	Zevenwacht	18°43′35″	33°55′16″	100
9	Mooiplaas	18°44′32″	33°55′29″	17
10	Wolfkloof 1	18°45′58″	33°54′53″	125
11	Wolfkloof 2	18°46′15″	33°55′17″	125
12	Koopmanskoop 2	18°46′58″	33°54′04″	7
13	Middlepos	18°38′37″	33°40′14″	4
14	Klipheuwel	18°41′23″	33°41′52″	52
15	Remshoogte 1	18°38′55″	33°38′33″	20
16	Helderfontein	18°42′52″	33°34′03″	100
17	Remshoogte 2	18°39′29″	33°38′51″	14
18	Klapmuts	18°44′45″	33°44′04″	34

Molecular analysis

DNA was isolated from 10 mg of dried plant material of individual plants using the CTAB (cetyltrimethylammonium bromide) method (Rogers & Bendich, 1994). Both DNA isolation and AFLP methods (Vos *et al.*, 1995) were adapted as previously described (Reisch, Anke & Röhl, 2005; Reisch, 2008). DNA concentration was estimated photometrically, and samples were standardized at a dilution of 7.8 ng/μl. For the AFLP procedure, genomic DNA (approximately 50 ng) was used for restriction and ligation reaction with MseI and EcoRI restriction enzymes and T4 DNA ligase (both Fermentas) conducted in a thermal cycler for 2 h at 37°C. Polymerase chain reactions (PCRs) were run in a reaction volume of 5 ml. Preselective amplifications were performed using primer pairs with a single selective nucleotide, MseI and EcoRI together with H_2O, Puffer S, dNTPs and Taq-Polymerase (Peqlab, Nürnberg, Germany). The PCR parameters were as follows: 2 min at 94°C, 30 cycles of 20 s of denaturing at 94°C, 30 s of annealing at 56°C and 2 min of extension at 72°C, followed by 2 min at 72°C and ending with 30 min at 60°C. After an extensive screening of selective primer combinations with eight randomly selected samples, selective amplifications were performed with each three primer combinations (*Hemimeris racemosa*: CTC-AAC, CTC-AAG, CTG-ACT; *Eriocephalus africanus*: CTC-AGC, CAG-AAG, CTG-ACT) and H_2O, dNTPs and Taq-Polymerase (PeqLab).

Polymerase chain reactions were performed with the touch-down profile: 2 min at 94°C, ten cycles of 20 s of denaturing at 94°C, 30 s of annealing, which was initiated at 66°C and then reduced by 1°C for the next ten cycles, 2 min of elongation at 72°C, followed by 25 cycles of 20 s of denaturing at 94°C, 30 s of annealing at 56°C and 2 min of elongation at 72°C, ending with a final extension for 30 min at 60°C. After DNA precipitation, DNA pellets were vacuum-dried and dissolved in a mixture of Sample Loading Solution and CEQ Size Standard 400 (both Beckman Coulter, Krefeld, Germany). The fluorescence-labelled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter). Raw data were collected and analysed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter). Data were exported as crv-files, showing synthetic gels with AFLP fragments for each primer combination separately from all studied individuals and analysed in BIONUMERICS (Applied Maths). Files were examined for strong, clearly defined bands. Each band was scored across all individuals as either present or absent.

Statistical analysis

In the AFLP data matrix, the presence of a band was scored as 1, whereas the absence of the band was coded as 0. Finally, basic data structure consisted of a binary (0/1) matrix, representing the scored AFLP markers. Genetic variation within populations was measured as Nei′s Gene Diversity, Shannon′s Information Index and percentage of polymorphic loci (PL) calculated in POPGENE v. 1.32 (Yeh *et al.*, 1997). Genetic variation between populations was measured using an analysis of molecular variance (AMOVA) (Excoffier, Smouse & Quattro, 1992) using GENALEX v. 6.2 (Peakall & Smouse, 2006). Based on the AMOVA measurements, we calculated sample size independent Sswp/n-1 values as an estimator for genetic variation within populations (Fischer & Matthies, 1998). A Mantel test, based on 999 permutations, was conducted to test whether the matrix of pairwise genetic distances (Φ_{PT}), taken from the AMOVA between populations, was correlated with the matrix of geographical distances between populations (Mantel, 1967). Finally, we used *t*-tests and Pearson correlations in PASW Statistics 17 (SPSS, IBM,

Armonk, NY, USA) for Windows to identify differences in genetic variation between the study species and to test whether genetic variation is related with renosterveld fragment size or distance.

Results

Amplified fragment length polymorphism analyses revealed 272 and 176 loci for *Hemimeris racemosa* and *Eriocephalus africanus*, respectively. The two species had the following mean genetic variation: $GD_{Hr} = 0.17$, $GD_{Ea} = 0.25$; $SI_{Hr} = 0.28$, $SI_{Ea} = 0.37$; $PL_{Hr} = 66\%$, $PL_{Ea} = 68\%$, $Sswp/n\text{-}1_{Hr} = 29.39$, $Sswp/n\text{-}1_{Ea} = 20.93$, respectively (Table 2). GD, SI and Sswp/n-1 were significantly higher in *E. africanus* (*t*-test, $P < 0.05$, GD: $t = -12.05$ $P = 0.001$, SI: $t = -0.83$, $P = 0.001$, Sswp/n-1: $t = 11.42$, $P = 0.000$). However, PL was at a similar level and not significantly different in both species (PL ~ 67%, *t*-test, $P < 0.05$, $t = -0.76$, $P = 0.46$). No correlation of fragment size and distance with genetic variation occurred (Table 3). Analyses of molecular variance (Table 4) showed similar low levels

Table 2 Genetic variation within populations of *Hemimeris racemosa* and *Eriocephalus africanus.* (Pop.: population number, N: sample size, GD: Nei's gene diversity, SI: Shannon's Index, PL: percentage of polymorphic loci, Sswp/n-1: AMOVA-derived index of within-population variation)

	Hemimeris racemosa					*Eriocephalus africanus*				
Pop	N	GD	SI	PL	Sswp/n-1	N	GD	SI	PL	Sswp/n-1
1	14	0.17	0.26	61.8	29.07	20	0.21	0.32	66.6	16.24
2	18	0.18	0.28	68.4	29.07	20	0.20	0.31	64.2	17.47
3	18	0.18	0.29	71.3	30.76	12	0.28	0.41	71.0	22.64
4	19	0.21	0.32	76.8	32.86	20	0.25	0.37	67.6	20.17
5	16	0.18	0.28	73.5	29.92	18	0.26	0.38	68.2	22.30
6	10	0.15	0.24	56.3	28.00	9	0.21	0.32	58.0	20.67
7	20	0.17	0.27	64.3	27.37	20	0.26	0.38	69.9	20.50
8	19	0.18	0.28	72.2	29.32	21	0.26	0.39	69.9	20.27
9	20	0.16	0.25	64.0	26.44	22	0.26	0.39	72.2	21.61
10	10	0.14	0.21	47.8	24.52	10	0.27	0.40	71.0	24.19
11	14	0.16	0.25	60.3	26.27	16	0.26	0.39	73.9	23.65
12	13	0.17	0.26	61.8	27.90	7	0.23	0.33	56.9	21.86
13	19	0.19	0.30	73.9	32.87	20	0.25	0.37	68.8	21.32
14	20	0.19	0.30	79.8	33.13	20	0.26	0.38	71.0	20.25
15	18	0.18	0.28	70.2	30.08	20	0.27	0.40	72.2	21.01
16	8	0.16	0.25	53.0	29.46	9	0.23	0.34	59.1	20.31
17	18	0.19	0.29	71.3	31.81	20	0.28	0.42	76.1	23.39
18	18	0.18	0.28	69.9	30.16	20	0.25	0.36	66.5	18.84
All populations		0.17 ± 0.01	0.27 ± 0.01	66.5 ± 1.9	29.39 ± 2.40		0.25 ± 0.01	0.37 ± 0.01	68.0 ± 1.2	20.93 ± 2.04

Table 3 Pearson correlation coefficient of fragment size and distance with genetic variation. No significant correlations occurred at $P < 0.05$ (*Hr*: *Hemimeris racemosa*. *Ea*: *Eriocephalus africanus*). For information of table legends. see Table 2

	Correlation of fragment size with				Correlation of mean distance to neighbouring remnants with			
	GD	SI	PL	Sswp/n-1	GD	SI	PL	Sswp/n-1
Hr	0.000	−0.032	−0.079	−0.076	0.310	−0.318	−0.451	−0.216
Ea	−0.256	−0.241	−0.012	−0.421	0.178	0.164	−0.057	0.275

Table 4 Analysis of molecular variance (AMOVA) of *Hemimeris racemosa* and *Eriocephalus africanus*. Significance level ($P > 0.001$) is based on 999 permutations. (I: Individuals. N: number of populations. Loci: number of AFLP fragments. df: degrees of freedom. SS: sums of squares. MS: mean squares. %: proportion of genetic variation. Hr: *Hemimeris racemosa*. Ea: *Eriocephalus africanus*)

Species	I	N	Loci	Genetic variation	df	SS	MS	%	Phi_{Pt}
Hr	292	18	272	Between populations	17	1453.73	85.51	10	0.10
				Within populations	274	8127.01	29.66	90	
Ea	304	18	176	Between populations	17	928.83	54.64	9	0.09
				Within populations	285	5899.73	20.70	91	

of genetic variation between populations for both *H. racemosa* (10%, $Phi_{Pt} = 0.10$) and *E. africanus* (9%, $Phi_{Pt} = 0.09$). A Mantel test (Figure 1) showed a significant isolation by distance for *H. racemosa* (N = 292, r = 0.71, $P = 0.001$) but not for *E. africanus* (N = 304, r = −0.04; $P = 0.39$).

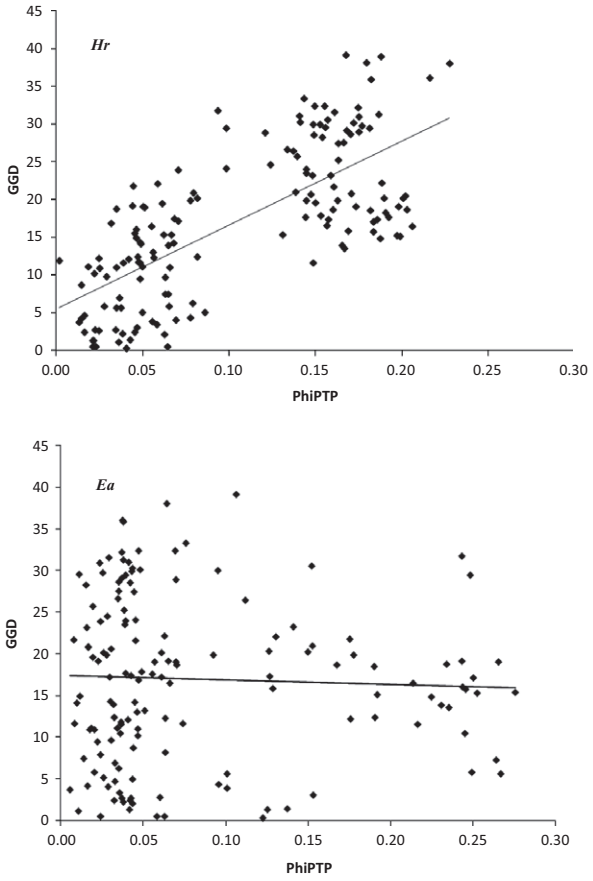

Fig 1 Correlation of genetic and geographic distances analysed in a Mantel test for *Hemimeris racemosa* (Hr: r = 0.71; $P = 0.001$) and *Eriocephalus africanus* (Ea: r = − 0.04; $P = 0.39$). GGD: geographical distance in kilometres. Genetic distance as PhiPT values

Discussion

Only few studies have analysed genetic variation of more than one species in the same spatial context (Apararcio *et al.*, 2012). It is well known that annual and insect-pollinated species exhibit lower levels of genetic variation within populations than perennial and wind-pollinated species (Hamrick & Godt, 1996). Our results support this observation, as genetic variation within populations of *Hemimeris racemosa* was significantly lower than that of *Eriocephalus africanus*. However, genetic variation within populations was within the range observed for other herbaceous annual and shrubby perennial species (Hamrick & Godt, 1996; Nybom & Bartish, 2000). We postulated that genetic variation in small populations of an annual species should be lower than in small populations of a perennial species, as the effects of drift and inbreeding are known to depend strongly on the length of the life cycle (Frankham, Ballou & Briscoe, 2002). However, unlike our original assumption, genetic variation within populations was not correlated with the size or distance between renosterveld fragments and variation was not more strongly decreased in *H. racemosa* compared to *E. africanus*.

Furthermore, genetic variation between populations was low compared to observations for species with similar life-history traits (Nybom & Bartish, 2000), but comparable to the results of other studies that focused on population distances smaller than 20 km (Hooftmann *et al.*, 2004; van Rossum, Campos De Sousa & Triest, 2004; Leimu & Mutikainen, 2005; Honnay *et al.*, 2006). However, genetic variation between populations of the annual was only marginally higher than between populations of the perennial species; both were comparably low.

The low level of genetic variation between populations indicates considerable historical or even current gene flow between populations.

However, both species exhibit a different spatial genetic pattern. While we observed a correlation of geographical and genetic distance between populations for *H. racemosa*, this relationship could not be detected for *E. africanus*. This may be traced back to the life-history traits of the species. The level and direction of pollen and seed dispersal is generally more restricted in an insect-than in a wind-pollinated species (Slatkin, 1985).

The observed differences in genetic variation between the selected species may therefore be attributed to the species life-history traits.

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